Structure of a Key Link in the Respiratory Chain

The Escherichia coli *Fumarate Reductase Respiratory Complex*

Even in bacteria, respiration is not a thing to be taken lightly. Though aerobes and anaerobes do it differently, the protein complexes involved in respiration are homologous across species, even from bacteria to humans. Thus, information about how a microbe "breathes" helps humans understand their own manner of generating energy in cells. Researchers working at the Macromolecular Crystallography Facility (MCF) at the ALS have grasped a big piece of the respiratory jigsaw puzzle by solving the structure of fumarate reductase, or respiratory complex II, from the bacterium *E. coli*. This protein is similar to succinate dehydrogenase, which catalyzes the oxidation of succinate to fumarate while functioning as complex II in the aerobic bacterial respiratory chain and also as a member of the Krebs cycle in eukaryotes. Fumarate reductase catalyzes the opposite reaction, reducing fumarate to succinate in anaerobic bacterial respiration. The two proteins are so similar that, under certain conditions, one can functionally replace the other.

The scientists, a group from the California Institue of Technology and San Francisco's Department of Veterans Affairs Medical Center, solved the structure of fumarate reductase to a resolution of 3.3 Å by using multiple-wavelength anomalous diffraction (MAD). They took advantage of the tunability of ALS wiggler light, collecting data sets at three different wavelengths near the iron K edge. The final model was a good fit with the diffraction data (R_{cryst} = 22.2% and R_{free} = 29.2%), particularly for a membrane protein, as these are traditionally difficult to solve. The intensity of the light from Beamline 5.0.2 gave a higher signal than would have been possible at most other crystallography facilities.

The new structure shows how the four subunits of fumarate reductase fit together. The subunits include a flavoprotein, an iron-sulfur protein, and two membrane anchor subunits that associate with quinone molecules. These four subunits turned out to be arranged in the shape of the letter "q", where the flavoprotein and the iron-sulfur protein comprise the circle, and the membrane anchors constitute the stem. The whole structure is very modular, with the flavoprotein subunit associating only with the iron-sulfur subunit, and the iron-sulfur subunit in turn associated with the two membrane anchors. The q-shaped complexes were found in pairs. These were associated through their membrane-anchor regions, yielding a shape like the letters "qp". Although this structure gives the initial appearance of being a dimer, the lack of physiological evidence to support dimerism and the short length of the contact region make it unlikely.

Since the crystals studied contained bound oxaloacetate, which inhibits fumarate reductase activity, the presence of the inhibitor near the flavoprotein subunit confirmed the flavoprotein as the active site for binding succinate or fumarate. The sequence of iron-sulfur clusters in the iron-sulfur protein subunit also confirmed previous studies, which predicted the 2Fe-2S moiety to be nearest the flavoprotein and the 3Fe-4S one to be nearest the membrane anchors. The membrane anchors were found to span a surprisingly long distance, putting the two quinones a physiologically unlikely 27 Å apart. Conducting electron transport over such a distance would be energetically difficult. The solution may be a cavity found nearly midway between the two quinone sites, serving as a way station only 13 Å from one quinone and 14 Å from the other.

The new information about fumarate reductase provides a framework for understanding the function of an entire family of proteins. The similarity of its subunits to other respiratory protein structures also gives intriguing clues to the functional origins of the protein. Together with recently solved structures within complexes III, IV, and V of the respiratory pathway, it gives a more complete picture of respiration, both with and without oxygen. ■

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T.M. Iverson, C. Luna-Chavez, G. Cecchini, and D.C. Rees, "Structure of the *Escherichia coli* Fumarate Reductase Respiratory Complex," *Science* **284** (1999), 1961–1966.

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STRUCTURE OF A KEY LINK IN THE RESPIRATORY CHAIN

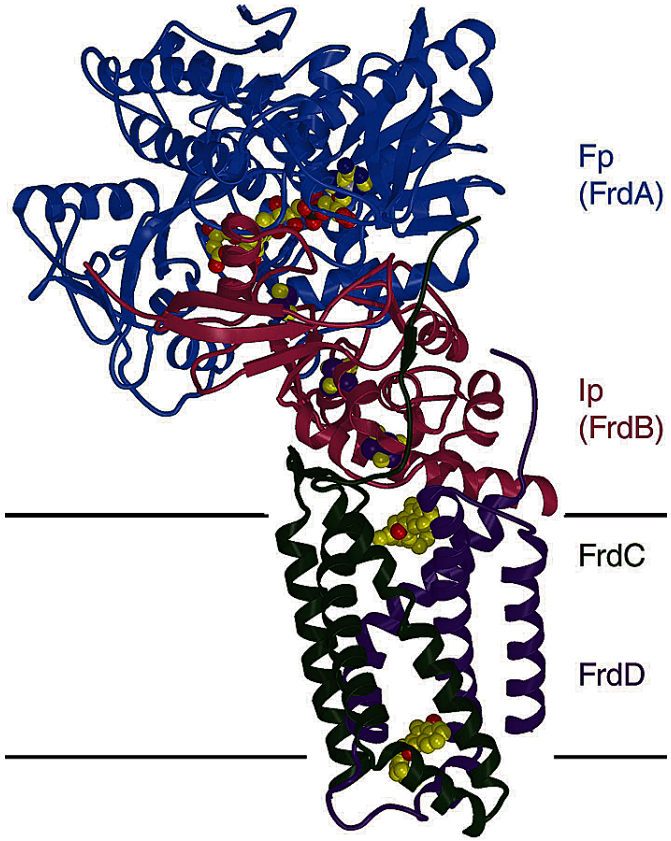
The Escherichia coli Fumarate Reductase Respiratory Complex

- **Fumarate reductase**
  - *Functions in anaerobic respiration when fumarate is the final electron acceptor*
  - *Homologous with succinate dehydrogenase*
  - *Key part of energy production in cells*
- **Studies at Macromolecular Crystallography Facility at the ALS**
  - *Multiple-wavelength anomalous diffraction (MAD) with wiggler light*
  - *Tunability used to obtain MAD data at three wavelengths near Fe K edge*
  - *Intensity of wiggler light yielded higher signal than possible at most other facilities*
- **Structure of the complex**
  - *Shows four modular subunits arranged in shape of letter "q"*
  - *Verifies binding site for FAD and ordering of FeS proteins*
  - *Shows that quinones attach far apart*
  - *Provides framework for understanding the function of a whole family of proteins*

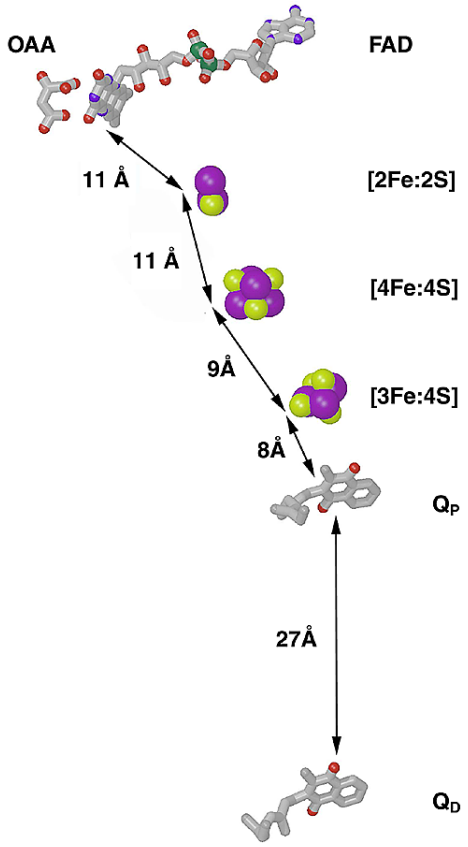
T.M. Iverson and D.C. Rees (California Institute of Technology) and C. Luna-Chavez and G. Cecchini (Department of Veterans Affairs Medical Center, San Francisco) [*Science* **284** (1999) 1961–1966].

STRUCTURE OF A KEY LINK IN THE RESPIRATORY CHAIN

The Escherichia coli Fumarate Reductase Respiratory Complex



Structure of the E. coli *fumarate reductase respiratory complex*



Spatial relationships between cofactors in the complex

T.M. Iverson and D.C. Rees (California Institute of Technology) and C. Luna-Chavez and G. Cecchini (Department of Veterans Affairs Medical Center, San Francisco) [*Science* **284** (1999) 1961–1966].